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Metal complexation with iodochlorhydroxyquin (clioquinol) targeting Aβ amyloid deposition and toxicity in Alzheimer's disease: proof-of-concept and safety.

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ABSTRACT

Background : The dementia of Alzheimer's disease (AD) is believed to be caused by the toxic accumulation of Aβ amyloid, due in part to excess binding of copper and zinc, metal ions that are abundant in the regions most affected. To test this Aβ amyloid theory of AD, we have developed a clinical intervention using a compound which binds copper and zinc, promotes the dissolution of aggregated Aβ, abrogates the toxic (H_2O_2-producing) properties of Aβ, and inhibits Aβ accumulation within the brain of a transgenic mouse model of AD.

Methods: Here we describe a Phase 2 clinical trial for the proof-of-concept for treatment of moderately severe AD patients, where the primary outcomes are efficacy (as measured by the ADAS-cog) and safety. Biochemical indicators include plasma Aβ amyloid levels and plasma zinc and copper levels.

Results: Thirty-six subjects were randomized [18 placebo and 18 clioquinol (CQ)]. Per protocol analyses were conducted on 32 subjects. The effect of treatment was statistically significant in the more-severely affected group (baseline ADAS-cog ≥ 25), but not the less-severely affected group (ADAS-cog < 25). The effect in the more-severely affected group was due to a substantial worsening of ADAS-cog scores in those taking placebo compared to negligible deterioration for the CQ group. Amongst the less-severely affected subjects, only minor worsening (not statistically significant) in ADAS-cog scores occurred in both placebo and CQ groups. Plasma $A\beta_{42}$ declined in the CQ group and increased in the placebo group. Plasma Zn levels rose by up to 30 per cent in the CQ-treated group. The drug was well tolerated by participants.

Interpretation: Subject to the usual caveats inherent in studies with small sample size, this phase 2 study has demonstrated proof-of-concept for a novel treatment strategy in AD. In subjects with

more-severe AD, there was little significant clinical progression after 36 weeks of treatment with CQ. Clinical efficacy was supported by biological changes underlying the etiology of $A\beta_{42}$ in Alzheimer's disease.

Key words: Alzheimer's disease, clinical trial, clioquinol, zinc, copper, Aβ amyloid.

There is now a general consensus on the theory that the causation of Alzheimer's disease (AD) lies within the pathway of the intracerebral biogenesis and accumulation of the Aβ amyloid protein (see recent reviews [1-2]). Although strongly supported by genetic and experimental evidence, the most convincing test of this theory will be the demonstration that drugs which target this pathway are efficacious in modifying the disease itself. Unravelling the proteolytic processing of the amyloid precursor protein (APP) which generates the Aβ amyloid has presented a number of therapeutic targets[3-5], and several of these approaches are at an early stage of clinical development. One such approach, immunization with Aβ to promote its clearance from the brain, has proven difficult with serious adverse effects[7].

We have developed a novel strategy of targeting the solubility and neurotoxicity of Aβ through disruption of Aβ-metal ion interactions. When Zn^{++} and Cu^{++} interact with Aβ, aggregation of Aβ into fibrils and plaques occurs[7-9]. At the same time, redox-active Cu^{++} -Aβ interactions can generate H_2O_2 from O_2 (ref 10). Both Cu^{++} and Zn^{++} can affect Aβ-lipid membrane interactions[11], a likely site for the toxic gain-of-function of Aβ. Compounds targeted to preventing Aβ-metal ion interactions should therefore have dual effects: promote solubilization of Aβ (and hence its clearance from the brain), and an abrogation of the Aβ-metal mediated toxic gain-of-function. One such lead compound, iodochlorhydroxyquin [PBT-1; an anti-infective agent also known as clioquinol (CQ)], a bioavailable Cu/Zn chelator, has been shown to promote the solubilization of AD plaque amyloid, to decrease the H_2O_2-generating capacity of Aβ, and cause a 49% decrease in brain Aβ deposition in a transgenic mouse model of AD[12].

CQ was withdrawn for oral use in 1970 due to its association with a rare neurological syndrome, subacute myelo-optic neuropathy (SMON), largely confined to Japan in the 1960s[13]. Recent

evidence suggests that SMON was caused by an overuse-related vitamin B_{12} deficiency [14] in an exceptionally vulnerable population, and therefore could be rehabilitated for study in a clinical setting[15]. A recent pilot study of CQ on AD patients treated with 20 mg and 80 mg/day for 20 days did not show any drug-related adverse effects[16].

On the basis of our preclinical data[12], we prepared a Phase 2 clinical trial of CQ for the treatment of AD. This Phase 2 study involved community dwelling patients with dementia. Because the primary outcome was efficacy, a double-blind design was chosen. A dose escalation schedule was chosen that would maximize the chance of detecting a cognitive change, whilst minimizing the risk of adverse effects. The starting dose of 3.3 mg/kg/day was within the same order of magnitude of the effective dose in the transgenic mouse model, but only about one tenth of the anti-infective dose.

In this manuscript, we report on the results demonstrating the possible disease-modifying effects of CQ (as measured by cognitive parameters and blood levels of Aβ) which provide the first human proof-of-concept evidence for the Aβ amyloid theory of AD.

METHODS

Ethical issues: In compliance with Australian laws concerning consent from individuals whose cognitive function may be impaired to the extent of being unable to make informed judgements or decisions, "Consent to Special Procedures" administered by the Victorian Civil and Administrative Tribunal was obtained for each participant not able to consent on their own behalf. In addition, third party consent was obtained from all carers. All subjects were stabilized

on donepezil prior to commencement of the study. The study was approved by the Royal Melbourne Hospital Research Foundation's Clinical Research and Ethics Committee.

Study population: The study took place at the AD clinical trials unit, Mental Health Research Institute of Victoria and at the Royal Melbourne Hospital. Criteria for inclusion in the study were: informed consent; a diagnosis of probable Alzheimer's disease by NINCDS-ADRDA criteria[17]; Alzheimer's Disease Assessment Scale-cognitive (ADAS-cog)[18] score of 20-45; Mini Mental State Examination (MMSE)[19] score of 10-24; on donepezil 5mg or 10mg for at least 6 months; relative or carer willing and able to support the trial; able to complete trial examinations; primary sensorial functions intact.

Patients were excluded if they had a history or clinical evidence of peripheral or optic neuropathy or had co-existing illnesses or past history that may have affected cognitive function, nerve conduction or illnesses that may have confounded the adverse event profile.

The following factors were obtained at baseline to determine if they correlated with outcome measures: age, sex, premorbid IQ [estimated from the National Adult Reading Test (NART)], years of education, and apolipoprotein E (ApoE) allotype .

Study design: The study was a double blind, placebo-controlled, parallel group randomized design. Thirty-six patients and their carers were recruited to participate, with patients randomized at a 1:1 ratio to receive either CQ or placebo. The duration of the study was 36 weeks. CQ oral dosage was 125mg bid from weeks 0-12, increased to 250mg bid from weeks 13-24, and finally, 375mg bid from weeks 25-36.

Study procedures: Screening procedures consisted of a full medical history, full physical, neurological and ophthalmic examination, blood and urine tests and psychometric tests (ADAS-cog, MMSE) to confirm the patient's eligibility for the study. Nerve conduction tests and visual evoked responses were conducted between the screening and baseline visits to provide a baseline measurement. Blood was collected for ApoE allotyping, baseline plasma levels of metals and Aβ prior to randomization. All patients continued their study entry dose of donepezil and all patients received 100 μg vitamin B_{12} intramuscularly every four weeks.

Outcome measures: The primary efficacy variable was a change from baseline score on the ADAS-cog conducted at baseline and at weeks 4, 12, 24 and 36. This measure was chosen to allow comparability of treatment effects with current therapeutics such as donepezil, where efficacy trials also used ADAS-cog as their primary outcome measure [20]. Although numerous neuropsychological tests could be considered as secondary measures, it was necessary to avoid fatiguing the subjects at review. Therefore the only other cognitive test was the Mini-Mental State Exam (MMSE). The CIBIC (clinician interview based impression of change incorporating caregiver information), a subjective observational index was also conducted. Plasma Aβ, and plasma zinc and copper were all taken 4 weekly.

Double antibody capture enzyme-linked immunosorbent assay (ELISA) for Aβ detection: Polystyrene plates were coated with mAb G210 (for Aβ40) or mAb G211 (for Aβ42). Plates were washed and biotinylated mAb WO2 was added. Bound antibody was detected with streptavidin-labelled Europium (Perkin Elmer, Vic Australia). The values obtained from triplicated wells were calculated based on standard curves generated on each plate.

Therapeutic drug monitoring: At weeks 12, 24 and 36, CQ blood levels were assayed by HPLC (Centre for Pharmaceutical Research, University of South Australia).

Safety measures: Standard adverse event reporting was conducted and biochemical tests, renal and liver function, full blood examination, serum vitamin B_{12} and folate levels were documented at each visit. To assess for peripheral and optic neuropathy a neurological examination was conducted at each visit, and visual evoked responses, nerve conduction studies and a full ophthalmic examination were conducted at screening, week 16 and prior to the final trial visit. An ECG was done at screening and weeks 12, 24 and 36.

Extension study: All patients who completed the double blind trial were invited to continue on a 48 week, prospective, open-label study of CQ which is still ongoing.

Data preparation and statistical analysis: Data monitoring and management were undertaken by independent contractors (Kendle International and Health Research Solutions, Melbourne). Evidence for efficacy was indicated by a significant difference in change from baseline between treatment arms. Analysis of variance was the principal method of evaluating statistical significance with the treatment arm illness severity at baseline being the primary design factor. Potentially significant covariates were introduced as necessary. Differences between groups on categorical measures were analysed using exact statistical methods in order to maximise power. Based on the assumption of a correlation of 0.60 between measurement occasions, power to detect an effect of one standard deviation difference in change between groups from baseline to week 36 would have been approximately 80% if 15 subjects were recruited per group. Since an attrition rate of 15% has been observed in similar populations, 18 patients were recruited into each arm.

The baseline illness severity factor was created, as planned, by division of the sample into two groups at the median ADAS-cog score at baseline, yielding less-severely and more-severely affected groups (n=8 and 8 in the treatment arm and n=7 and 9 in the placebo arm, respectively).

Role of funding sources: The basic research and assays required for this study were developed in part from support from the NH&MRC and the Baxter Trust. The clinical trial itself was supported in part by Prana Biotechnology.

RESULTS:

Subject recruitment and demographics: Thirty six subjects were recruited over a 12 month period commencing April 2000 (Fig 1). Of these, 32 had sufficient data for per protocol analysis. Two subjects were lost from each arm. In the placebo arm, one subject died and another withdrew because of illness unrelated to AD. In the treatment arm, one subject withdrew because of behavioral changes associated with AD (paranoia, leading to non-cooperation with testing). Another subject was not included in the analysis because their initial diagnosis of AD was probably incorrect (the symptoms and signs evolved into a picture characteristic of Diffuse Lewy Body disease). Exclusion of this subject had no effect on the outcomes of this study. A third subject withdrew at week 24 due to unrelated medical problems. Data for this subject were included in all analyses except those involving week 36. Five subjects (three in treatment and two in placebo arms) did not tolerate the final dosage increase (375mg bid) and were returned to the previously tolerated dose (250mg bid) for the remainder of their involvement in the study.

The groups did not differ across all demographic, biological and clinical parameters at baseline (Table 1), other than the treatment arm having a higher mean premorbid IQ than the placebo group as estimated using the NART (111.4 compared to 104.9; $t(30)=2.27$, $p=0.031$) and a lower level of thyroid stimulating hormone (TSH) (1.14 compared to 2.00 mU/L; $t(30)=4.400$, $p<0.001$). The NART and TSH were subsequently provisionally entered into analyses as co-variates but were found to be not significant in any analysis.

Proof-of-concept: Ideally, any drug targeting the Aβ amyloid pathway should have two principal effects: a disease-modifying effect as assessed by cognitive parameters and a biological response assessed by measurement of Aβ in blood, CSF or the brain itself.

Changes in the ADAS-cog score at weeks 4, 12, 24 and 36 from baseline were subject to two-way analysis of variance with factors of treatment arm and baseline illness severity. As planned in the protocol, the effect of severity of illness was examined by stratification of the sample into subjects less- or more- severely affected than the median value of the base line ADAS-cog (values <25, ≥ 25). At baseline there were no significant or near-significant differences between the main effect of treatment arm [$F(1,28)=0.21$, $p=0.647$]. Similarly, there were no significant differences between treatment arms at either level of severity. The main effect of treatment arm was not significant at any week, although trends toward significance were noted at week 4 [$F(1,28)=3.55$, $p=0.070$] and week 24 [$(F(1,28)=3.31$, $p=0.080$] (Fig 2A). Simple effects tests within level of severity showed the main effect to be separable into non-significant results for the less-severe stratum on all weeks and significant differences in the more-severe stratum at weeks 4 [$F(1,28)=7.73$, $p=0.010$] and week 24 [$F(1,28)=6.63$, $p=0.016$] (Fig2B). This trend was maintained at week 36 but marginally escaped statistical significance [$F(1,28)=3.62$, $p=0.068$]. The difference in mean change from baseline ADAS-cog score of CQ over placebo at weeks 24

and 36 was a difference of 6.36 (95% CI: -0.50 – 13.23) and 7.37 (95% CI: 1.51 – 13.24) respectively.

The MMSE, a less sensitive measure of cognitive impairment, showed a similar pattern without reaching significance. By contrast, ADAS – noncog and CIBIC did not show any clear differences or trends. ApoE genotyping did not disclose any effect other than an over-representation of the ε4 allotype in both groups.

There were no significant differences in baseline plasma $A\beta_{42}$ levels between treatment arms or severity strata. Plasma $A\beta_{42}$ showed a significant decline from baseline in the CQ-treated group from week 20 onwards; over the same time, plasma $A\beta_{42}$ in the placebo group increased (Fig 3A). Stratification by illness severity as above demonstrated that changes were evident only in the less-severely affected (Fig 3B). The wide variance in individual levels at baseline in plasma $A\beta_{40/42}$ led to reduced power of the study to detect any significant differences in mean changes between groups. The relative stability of individual $A\beta_{42}$ values therefore emerges as a potent window on cerebral Aβ metabolism.

Analysis of plasma $A\beta_{40}$ levels showed overall similar trends, with significant differences between placebo and CQ groups observed at weeks 8, 32, and 36 in the less-severely affected groups. For individuals, there was a highly significant ($p< 0.0001$) correlation between $A\beta_{42}$ and $A\beta_{40}$ levels.

Effect on plasma Zn and Cu: Administration of CQ was associated with a significant elevation of total plasma Zn (Fig 4A) but with no effect on plasma Cu (Fig 4B). Samples collected with an

indwelling catheter at weeks 12, 24 and 36 were found to be unreliable for technical reasons and were therefore omitted from this analysis (the metal levels in blood collected by this technique were depressed, compared to samples taken on other visits by single vein puncture, probably as a result of platelet activation). Mean absolute levels of Zn (9.4 μM) in all groups at baseline were below age-related normative values[21]. Mean absolute levels of Cu (13.1 μM) were within the age-related normative range[22]. Correlation of plasma $A\beta_{42/40}$ levels with Zn/Cu levels assayed on the same or subsequent occasions showed no significant associations.

Blood levels of CQ: Steady state pre-dose levels of CQ at total daily dosages of 250, 500 and 750 mg were 4.03±2.10, 6.74±3.70, 7.60±2.15 μg/ml, respectively, and did not show significant correlations with ADAS-cog, metal levels or Aβ levels assayed on the same or subsequent occasions.

Safety results and analysis: Safety analysis was conducted on all data irrespective of the stage reached in the trial. There were a total of 111 attributable adverse events (AE) reported, 61 in the treatment group and 50 in the placebo arm. The mean number of discrete events per subject was not significantly different between arms (Table 2). Five subjects developed serious adverse events (SAE). A 66-year-old female with hypertension and hyperlipidemia developed impaired visual acuity and color vision after having completed the trial and receiving CQ 375mg bid. This was considered to be probably attributable to CQ and her symptoms rapidly resolved upon its cessation. Four non-attributable SAE were recorded. There was one death due to intracranial hemorrhage (placebo) and three hospitalizations: for hip pain (placebo), syncope due to impaired cardiac function (CQ) and confusion (placebo).

Cardiac safety: Symptoms of postural cardiac insufficiency were common with 27/36 (75%) of subjects reporting this on at least one occasion. There were no significant between-group differences (Table 2).

Neurological safety: The development of new or worsening neurological symptoms or signs were uncommon. Analyses of the nerve conduction data revealed that there were no relevant or significant between-group differences. There were also no significant differences in the rates of impairment or deterioration in visual acuity, color vision, visual fields or of fundoscopic abnormalities between the two groups (using McNemar's test for paired categorical data).

Gastrointestinal safety: Subjects on CQ experienced fewer occurrences of diarrhea but had some changes in liver function tests (LFT). In the CQ arm, within-group analyses of γ-GT, AST and ALT showed intermittent significant elevations from baseline at various time points which were normalising by week 36. The only significant between-group difference occurred with γ-GT. No subject developed any overt symptoms or signs of impaired liver function.

Hematological safety: There were no hematological AE noted in the CQ treated subjects. However, there was a significant reduction in hemoglobin in the CQ arm over the course of the study, which by week 36 was 9.6 g/L ($F(1,27) = 6.135$, $P = 0.02$). Between-group differences were also observed at weeks 24 and 36. Corresponding changes in packed cell volume and red cell count were observed. The cause of these clinically insignificant decreases was uncertain. For all other hematological parameters, there were no significant between-group differences.

Renal safety: Serum creatinine was mildly elevated in both arms over the course of the study and a small significant drop in serum albumin was noted in the CQ arm, however there were no

significant between-group differences in these parameters. Serum urea remained unchanged in the placebo arm, and increased slightly in the CQ arm at weeks 4 and 8 with between-group analysis showing significant differences at weeks 12 and 36.

DISCUSSION

The data are offered as a proof-of-concept that a low molecular weight compound (CQ, mass 306d) targeting the Aβ pathway can have a significant disease-modifying effect on the natural history of AD. As such, these data are the first of what will hopefully become a series of independent observations, using other therapeutic strategies, confirming the Aβ amyloid theory of AD. The benefit of CQ in this study population was only seen in the more-severely affected subjects, probably due to the low power of the study and the limited sensitivity of the ADAS-cog to detect subtle cognitive differences in the less-severely affected groups over the nine month study period. It is worth noting that the statistically significant separation of 3 ADAS-cog points achieved after 24 weeks treatment with the acetylcholinesterase inhibitor, donepezil (Aricept), required a study population of more than 300 subjects[20]. The present study with an order-of-magnitude smaller population size, underscores the potential impact of the 7 ADAS-cog points difference observed in our study at 24 weeks (Fig1) if replicated in a future, larger, trial of CQ. The significant benefit seen in the more-severely affected treatment group at 4 weeks is also of interest, as this may represent the short-term effect of CQ neutralizing the neurotoxicity of the soluble pool of Aβ (less than 1% of the total Aβ burden[23]), a pool which is also accessible through immunomodulatory interventions[24]. Such a rapid clinical effect would also be consistent with that reported for CQ in a pilot, 3 week, open-label study at comparatively low dosage[16].

Perhaps more convincing than the cognitive effects of CQ are the data which show a lowering of plasma $A\beta_{42}$. Previous cross-sectional assays of blood Aβ levels have been disappointing because of large inter-individual variations[25], although one longitudinal study of pre-clinical AD disclosed higher plasma $A\beta_{42}$ levels[26], and within-pedigree measurements of plasma $A\beta_{42}$ in late onset AD have indicated a strong genetic effect[27]. The present longitudinal study appears to be the first to have followed affected individuals at regular intervals over an extended period, disclosing a progressive increase in plasma $A\beta_{42}$, principally in the less-severely affected group. There was a trend over time towards lower mean levels in the more-severely affected group (data not shown), which is consistent with the large body of data on CSF Aβ levels which show an elevation early in the disease, followed by a progressive fall as the disease evolves[28]. A 24% decrease in serum Aβ was also observed in CQ –treated Tg2576 mice models of AD[12]. The most parsimonious explanation of these observations is that Aβ is being sequestered into cerebral extracellular spaces as the disease unfolds. The pool of Aβ in equilibrium with the CSF may be different from that in the blood. Both blood and CSF Aβ pools could have non-cerebral contributions (for example, from platelets in the blood[29] and the choroid plexus in the CSF[30]) yet it would appear from the present data that plasma Aβ may yet emerge as a reliable surrogate marker for cerebral Aβ. The unexpected rapid turnover and efflux into plasma of cerebral Aβ in experimental animals[31] would suggest that active clearance/ degradative mechanisms are operating.

>From current experimental data, we surmise that CQ may be having at least two principal therapeutic effects on Aβ: promoting its zinc-dependent solubilization and hence clearance/degradation from the brain and also diminishing the toxic gain-of-function mediated by Aβ-copper interactions. The 30% increase in plasma Zn (from a below-normative baseline level)

associated with CQ treatment might arise from an exchangeable pool, whereas the lack of effect on blood Cu levels might reflect a more rigidly controlled metabolic pool. Experimental studies of CQ on mouse brain Zn and Cu levels showed increases of 13% (Zn) and 19% (Cu) in the soluble fractions[12]. Given the uncertainty in the proposed mechanism of action of CQ, it is difficult to predict *a priori* what an effective therapeutic dose might be. Experimentally, a molar ratio of $[CQ]_{EC}$:$[Metal]_{free}$: $[A\beta]_{sol}$ of at least 4:2:1 (where $[CQ]_{EC}$ is the effective extracellular concentration of CQ in the brain, $[Metal]_{free}$ is the concentration of free metals such as zinc ions in the peri-synaptic spaces of the glutamatergically-innervated cerebral cortex and hippocampus, and $[A\beta]_{Sol}$ is the concentration of the soluble "toxic" species of Aβ in the same extracellular cerebral compartment) should be required for the short- and longer-term neutralization of H_2O_2 production. The measured basal levels of plasma CQ in the current study ranged from 13 to 25μM. Allowing for a large proportion of CQ being bound to serum protein (albumin) or lipoproteins[32], the available active compound in the brain should be approximately 100-200 nM. The concentration of total Aβ in the AD brain varies considerably, but is estimated to range from the low nM to low μM range[23, 33], of which less than 1% is available as a "toxic" soluble species[23]. Actual measurements of brain $[CQ]_{EC}$ will be required, together with plasma pharmacokinetics, before a more rational approach to dosing can be applied, but the current available data suggest that the dosages employed in this study may be within a theoretical optimum. Other small drugs targeting the systemic amyloid pathway have been shown to operate with an experimentally determined stoichiometry of 5:2(ref 34), but have been used in human trials at much higher doses (40mg/kg/day) than those employed in the present study.

Safety issues were always of paramount importance in a study involving the chronic administration of a drug with a known history of adverse events. We balanced the risk of treating a malignant disease such as AD against the relatively low risk of developing SMON by careful

regular evaluations, ensuring complete normality of vitamin B_{12} and folate metabolism, and a preparedness to withdraw subjects at the first sign of SMON. CQ had no overall effect on nerve conduction, either optic or peripheral. Optic neuropathy was suspected in one subject, whose visual symptoms resolved rapidly upon treatment cessation. Objective measures of optic nerve conduction demonstrated bilateral decreased amplitude in visual evoked potentials at week 24 with further decrease at week 36. CQ may have been associated with optic neuropathy in this case, though evidence of a direct causal link between CQ and optic neuropathy is uncertain based on the results from this study. Disturbances of color vision and other ophthalmologic changes are known to occur during the natural history of AD [36-38].

The association between CQ and transient elevation of transaminases at week 24 may represent enzyme induction, with subsequent regression. The asymptomatic decrease in hemoglobin, where all subjects remained within the normal range, without changes to any other hematological parameter may reflect hemolysis or depletion of iron stores. Mild changes in renal function are unlikely to reflect significant nephrotoxicity of this drug. Further studies will be required to elucidate the mechanisms underlying all of these biochemical changes.

At the time of writing, 27 subjects had agreed to participate in an open-label extension study of CQ. More than 10 subjects have now been on this drug at 750 mg per day for more than 9 months. None has developed any CQ-attributable adverse events (this cohort will be the subject of a future publication). We conclude that the safety profile and apparent efficacy of CQ in this population are sufficiently encouraging to allow future trials to take this investigation of a novel therapeutic intervention, targeting Aβ amyloid, to the next phase.

Author contributions:

Study concept and design: C Ritchie, A Mackinnon, M Mastwyk, M Xilinas, D Ames, S Davis, C Masters; Acquisition of data: S Macfarlane, M Mastwyk, L MacGregor, L Kiers, R Cherny, QX Li, A Tammer, D Carrington, C Mavros, I Volitakis; Analysis and interpretation of data: C Ritchie, A Mackinnon, S Macfarlane, M Mastwyk, R Cherny, QX Li; Drafting of manuscript: C Masters; Critical revision of manuscript for important intellectual input: C Ritchie, A Mackinnon, S Macfarlane, D Ames, S Davis, K Beyreuther, C Masters; Statistical expertise: A Mackinnon; Administrative, technical and material support: M Mastwyk, L MacGregor, R Cherny, A Tammer, D Carrington, C Mavros, I Volitakis; Study supervision: S Macfarlane, M Mastwyk, C Masters.

Conflict of Interest Disclosure: Dr Craig Ritchie has been paid for his time and effort in analyzing the results from the trial sponsor, Prana Biotechnology. Dr Steve Macfarlance, Ms Maree Mastwyk and Dr Lachlan MacGregor were employed by the Mental Health Research Institute on funds provided by Prana Biotechnology. Prof Colin Masters is a Director of Prana Biotechnology, Chair of its Scientific Advisory committee, and a minor stockholder. Dr Robert Cherny is a minor stockholder in Prana Biotechnology. Professor Konrad Beyreuther is on the Scientific Advisory Committee of Prana Biotechnology.

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performing the CIBIC, Drs K Barnham, D Darby, P Desmond, and K Taubman for advice and consulting, and Ms F Fraser for assistance.

Legends

Fig 1

Outline of the flow chart of subjects studied.

Fig 2

Mean change (±SE) over time from baseline in cognitive abilities (as assessed with ADAS-cog) in (A) two arms of CQ vs placebo and (B) stratification by severity within treatment arms [less-severely affected (ADAS-cog < 25), more-severely affected (ADAS-cog ≥25) (*$p \leq 0.05$; ** $p \leq 0.01$).

Fig 3

Mean change (±SE) over time from baseline in plasma $A\beta_{42}$ levels in (A) the arms of CQ vs placebo and (B) stratification by severity as in Fig 2. (***$p \leq 0.001$)

Fig 4

Mean change (±SE) over time from baseline in (A) plasma Zn (B) plasma Cu in the two arms of CQ vs placebo.

TABLE 1. Baseline demographics and key clinical variables

Variable	Total Sample (n=32)	Group: Clioquinol (n=16)	Group: Placebo (n=16)	P Value
Age mean (SD; min-max)	72.50 (8.37; 56-87)	73.19 (8.61; 58-87)	71.81 (8.35; 56-87)	P=0.65†
Sex (n; % male)	17 (53.1%)	8 (47.1%)	9 (52.9%)	P=1.00‡
ApoE status				
ApoE4 heterozygote n (%)	15 (46.9%)	7 (43.8%)	8 (50.0%)	P=1.00‡
ApoE4 homozygote n (%)	3 (9.4%)	2 (12.5%)	1 (6.3%)	
Estimated premorbid IQ NART mean, (SD; min-max)	108.1 (8.86; 91-124)	111.4 (8.04; 94-121)	104.9 (8.26; 91-124)	P=0.03†
ADAS-Cog	26.31 (7.27; 15-46)	25.56 (7.67; 15-46)	27.06 (7.01; 19-41)	P=0.57†
Age of first diagnosis mean, (SD; min-max)	70.09 (7.98; 54-83)	70.88 (8.50; 57-83)	69.31 (7.61; 54-83)	P=0.59†
Duration of illness (years) mean (SD; min-max)	2.41 (1.19; 1-5)	2.31 (1.08; 1-4)	2.56 (1.32; 1-5)	P=0.66†

† Independent sample t-test (all tests 30 *df*)
‡ Exact, two-tailed test.

TABLE 2. ATTRIBUTABLE ADVERSE EVENTS WITH A RISK OF GREATER THAN 10% IN EITHER ARM OR WHERE POINT ESTIMATE RISK RATIO IS GREATER THAN 2.0 OR LESS THAN 0.5

	Treatment (n=16)	Placebo (n=16)	Relative Risk (95% CI)
Cardiovascular			
Postural hypotension	12	11	1.09 (0.67-1.79)
Postural tachycardia	12	8	1.33 (0.74-2.40)
Postural dizziness	7	3	2.33 (0.71-7.63)
Subjects with ≥1 postural symptom	13	14	0.93 (0.64-1.36)
Neurological			
Impaired nerve conduction	3	1	3.0 (0.34-26.2)
Impaired reflexes	1	2	0.5 (0.05-5.04)
Numb legs	2	0	-
Subjects with ≥1 symptom	6	4	1.5 (0.51-4.43)
Gastrointestinal			
Diarrhea	1	4	0.25 (0.03-2.02)--2.0 (0.2-
Constipation	2	0	20.1)1.25 (0.4-3.91)
Nausea	2	0	
Abdominal pain	2	1	
Subjects with ≥ 1 symptom	5	4	
RenalMicroalbuminuria			
	5	5	1.00 (0.35-2.87)
HematologicalLymphopenia			
	0	3	-
Liver Function Tests Raised γGTRaised			
bilirubin**Subjects with ≥1 abnormal**	2	1	2.0 (0.2-20.1)-
	2	0	4.0 (0.49-32.4)
	4	1	
Other			
Decreased vitamin B_{12}	0	2	-
Mean number of discrete adverse events per subject (SD)	3.38 (2.14)	2.78 (1.48)	Mean diff 0.611 (95% CI -0.64 - 1.89 p=0.327)

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Figure 1

Subject Accountability

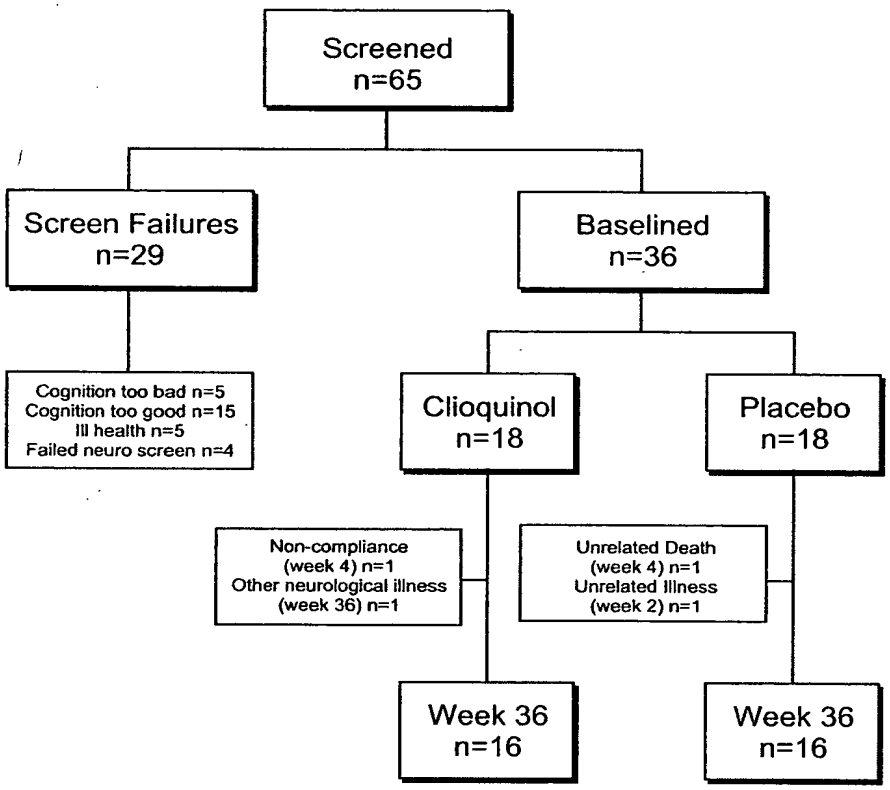


Figure 2

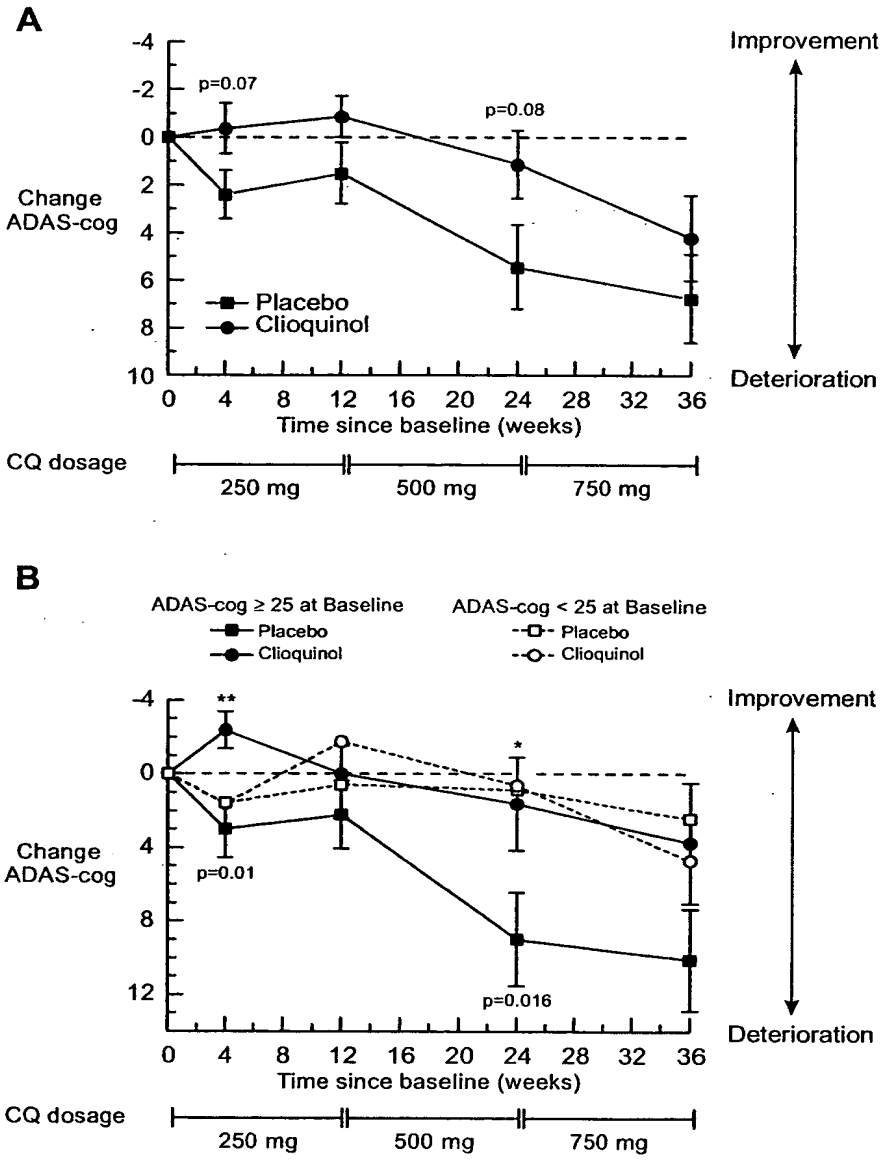

A
-4
-2
0
2
4
6
8
10
p=0.07
p=0.08
Change
ADAS-cog
Placebo
Clioquinol
Improvement
Deterioration
0 4 8 12 16 20 24 28 32 36
Time since baseline (weeks)
CQ dosage
250 mg
500 mg
750 mg
B
ADAS-cog ≥ 25 at Baseline
Placebo
Clioquinol
ADAS-cog < 25 at Baseline
Placebo
Clioquinol
-4
0
4
8
12
**
*
p=0.01
p=0.016
Change
ADAS-cog
Improvement
Deterioration
0 4 8 12 16 20 24 28 32 36
Time since baseline (weeks)
CQ dosage
250 mg
500 mg
750 mg

Figure 3

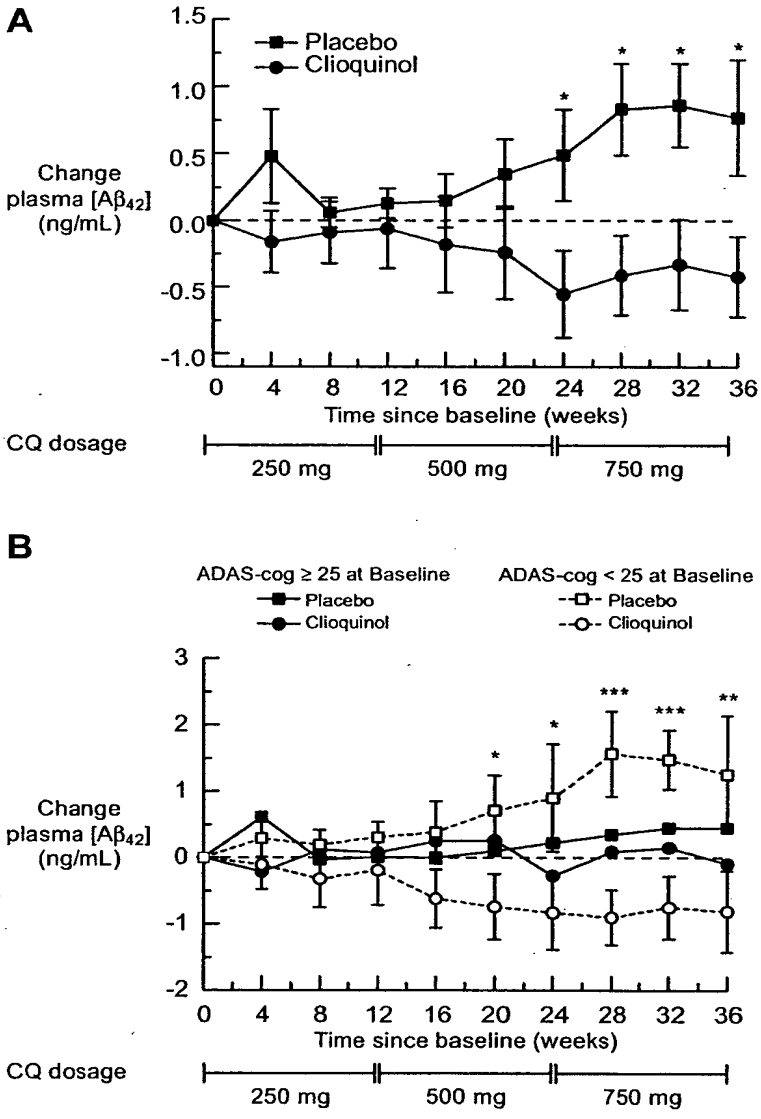
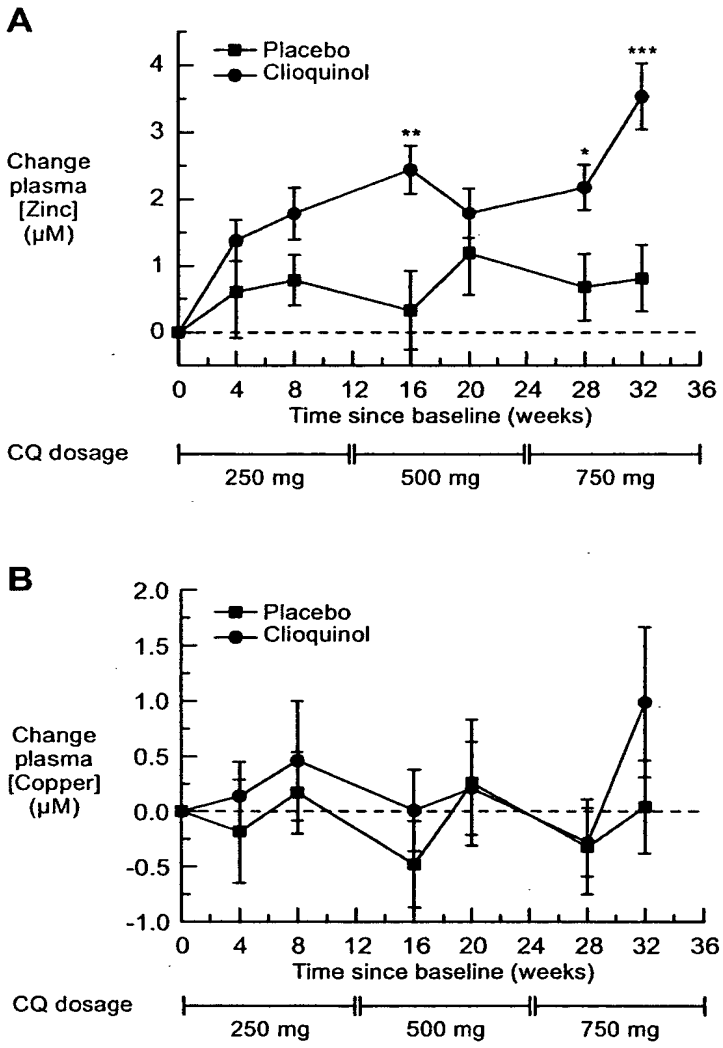

A
Placebo
Clioquinol
Change plasma [Aβ42] (ng/mL)
1.5
1.0
0.5
0.0
-0.5
-1.0
0 4 8 12 16 20 24 28 32 36
Time since baseline (weeks)
CQ dosage
250 mg
500 mg
750 mg
B
ADAS-cog ≥ 25 at Baseline
Placebo
Clioquinol
ADAS-cog < 25 at Baseline
Placebo
Clioquinol
Change plasma [Aβ42] (ng/mL)
3
2
1
0
-1
-2
0 4 8 12 16 20 24 28 32 36
Time since baseline (weeks)
CQ dosage
250 mg
500 mg
750 mg

Figure 4


A
Placebo
Clioquinol
Change plasma [Zinc] (μM)
4
3
2
1
0
**
*
***
0 4 8 12 16 20 24 28 32 36
Time since baseline (weeks)
CQ dosage
250 mg
500 mg
750 mg
B
Placebo
Clioquinol
Change plasma [Copper] (μM)
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
0 4 8 12 16 20 24 28 32 36
Time since baseline (weeks)
CQ dosage
250 mg
500 mg
750 mg